Comparative Analysis of Tissue Reactions to Anesthetic Solutions: Histological Analysis in Subcutaneous Tissue of Rats

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Postanesthetic pain is a relatively common complication after local anesthesia. This complication may be caused by the anesthetic technique or by the anesthetic solution used. Tissue reactions induced by the anesthetic solutions may be one of the factors resulting in pain after anesthesia. The objective of this study was to comparatively analyze tissue reactions induced by different anesthetic solutions in the subcutaneous tissue of rats. The following solutions were utilized: 2% lidocaine without vasoconstrictor; a 0.5% bupivacaine solution with 1 : 200,000 adrenaline; a 4% articaine solution and 2% mepivacaine, both with 1 : 100,000 adrenaline; and a 0.9% sodium chloride solution as a control. Sterilized absorbent paper cones packed inside polyethylene tubes were soaked in the solutions and implanted in the subcutaneous region. The sacrifice periods were 1, 2, 5, and 10 days after surgery. The specimens were prepared and stained with hematoxylin and eosin for histological analysis. The results showed that there is a difference in tissue irritability produced by the local anesthetic solutions. The results also showed that there is no relation between the concentration of the drug and the inflammatory intensity, that the mepivacaine and articaine solutions promoted less inflammatory reaction than the bupivacaine, and that the lidocaine solution produced the least intense inflammation.

Key words: Local anesthetics; Biocompatibility; Mepivacaine; Bupivacaine; Articaine; Lidocaine.

Local anesthesia is the most routinely used dental procedure in daily practice.[1] The introduction and development of procaine by Einhorn in 1904 revolutionized pain control in dentistry and medicine.[2] From this starting point, dental anesthesiology underwent accentuated progress, in techniques as well as in the solutions utilized for anesthesia.[3–11]

Today, anesthetic solutions are relatively secure and standardized for use; however, the solutions are still being studied with the intent of developing substances with more controlled tissue effects, improving the effectiveness and reducing the side effects of these drugs.[12–15]

These solutions are not selective drugs, and they can interfere significantly in the homeostasis at the point where they are injected,[12–14] as well as systemically.[15–21] Local tissue irritation resulting in an inflammatory reaction, and consequently pain, during and/or after anesthesia can be observed in the utilization of some anesthetics.[22–26]

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Figure 1. Insertion of the polyethylene tube in the subcutaneous region.

In 1999, Kramp et al[24] evaluated pain reduction during and after the administration of intra-oral local anesthesia. They used a 4% prilocaine solution without vasoconstrictor, a 2% lidocaine solution with 1 : 100,000 adrenaline, and a 2% mepivacaine solution with 1 : 20,000 levonordefrine. With regard to the point of application, there was no difference; however, with regard to the solution that was applied, prilocaine proved to cause less pain when compared with lidocaine and mepivacaine.

The different tissue reactions induced by the anesthetics were also experimentally analyzed. In 1972, Benoit and Belt[14] tested the effects of some local anesthetics on the skeletal musculature in rats, whereby the different tissue reactions produced by the anesthetics were ascertained. In 1976, Carvalho et al[13] verified the subcutaneous tissue reaction in rats using the implantation of polyethylene tubes containing absorbent paper cones soaked in different anesthetic solutions. The authors were able to verify that the intensity of the inflammatory reaction of the subcutaneous connective tissue varied according to the type of anesthetic solution that was used. Lidocaine produced the mildest inflammatory reaction.

Considering the nonselectivity of local anesthetic molecules, and knowing that the degree of systemic toxicity does not always coincide with local tissue irritability induced by the anesthetic solution or with its strength,[17] we propose to evaluate the tissue reactions induced by some of these medications.

MATERIALS AND METHOD

Forty male rats (*Rattus norvegicus, Albinus wistar*) weighing 120–130 g were used. They underwent general anesthesia with a subcutaneous injection of a Thiopental sodium[1] solution (Thionembutal, Abbott Laboratories, São Paulo, Brazil). After trichotomy and antisepsis of the animal's dorsal region, two cutaneous incisions of approximately 1 cm were made at the median sagittal region, maintaining a distance between the cuts of 1.5 cm. At the upper incision, a divulsion of the subcutaneous incision toward the right side of the animal was performed, until sufficient space was created to install the polyethylene tube. At the lower incision, a divulsion of the tissue was performed in the opposite direction with the same depth.

The animals were divided into 5 groups with 8 rats each. One-centimeter long polyethylene tubes were implanted (Nasogastric probe #4, Embramed, São Paulo, Brazil). Absorbent paper cones (#30), 1 cm in length, were adapted to the interior of the tubes, and afterward these were soaked in the different tested anesthetic solutions.

The tested solutions were divided into 5 groups: (a) Group I (saline), 0.9% sodium chloride solution (Ariston Laboratory, São Paulo, Brazil); (b) Group II (bupivacaine), 0.5% bupivacaine hydrochloride solution, with 1 : 200,000 adrenaline (0.5% Neocaine, Cristália Laboratory, Itapira, Brazil); (c) Group III, (articaine), 4% articaine hydrochloride solution with 1 : 100,000 adrenaline (Septanest with 1 : 100,000 adrenaline, Septodont Laboratory, Saint-Maur-des-Fossés Cedex, France); (d) Group IV (lidocaine), 2% lidocaine hydrochloride solution, without vasoconstrictor (2% Xilocaine, Astra Laboratory, Naucalpan, Mexico); and (e) Group V (mepivacaine), 2% mepivacaine hydrochloride solution with 1 : 100,000 adrenaline (2% Special Scandicaine, Septodont Laboratory, Saint-Maur-des-Fossés Cedex, France).

The tubes were introduced into the subcutaneous space and positioned approximately 2 cm from where the incision was made (Figure 1). Afterward, the surgical wounds were sutured with 3-0 silk thread (Ethicon, Saõ José dos Campos, Brazil).

Two animals of each group were sacrificed by means

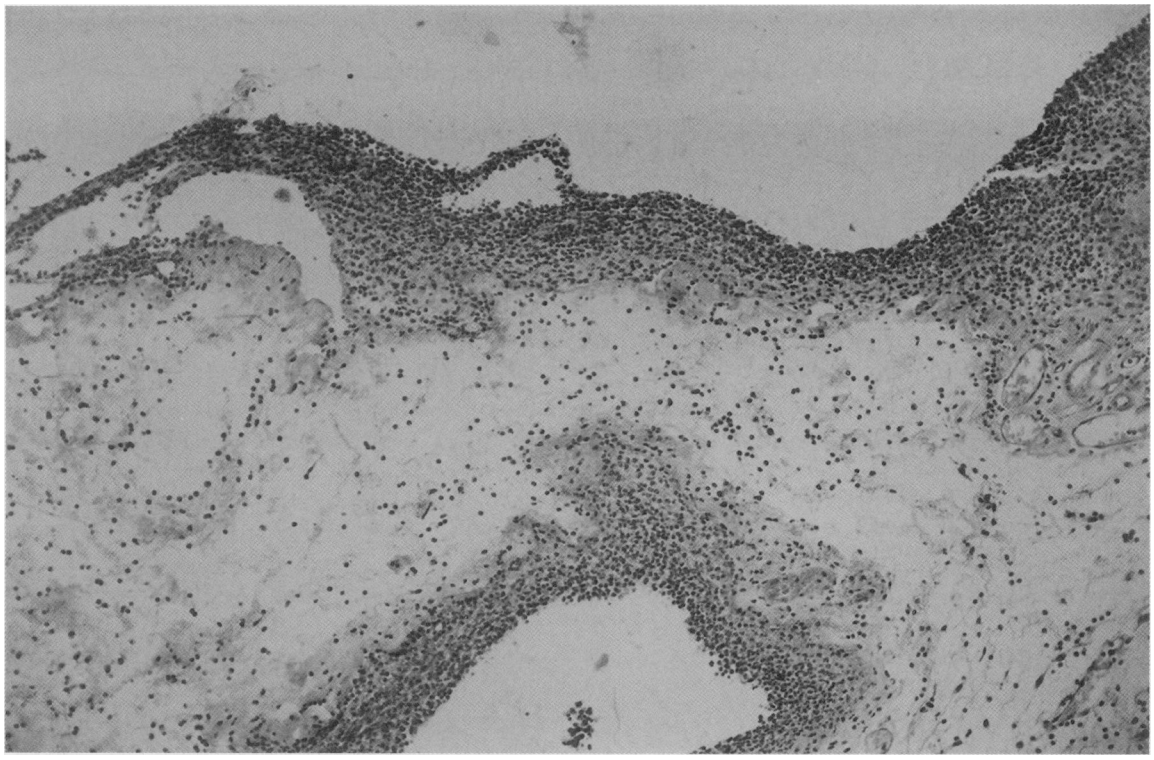

Figure 2. Group II (bupivacaine). 1 day. An elevated number of neutrophils in the superficial and deep areas was observed (HE; original magnification ×63).

of sulfuric ether inhalation, within a period of 1, 2, 5, and 10 days after implantation of the polyethylene tubes.

The specimens were removed and placed in recipients containing a 10% formalin solution for a period of 24 hours for fixation. Six-micrometer-thick semi-serial slices were made and stained with hematoxylin and eosin for conducting histological studies.

For the analysis of the reactions produced by the different implanted solutions, the Wolson and Seltzer[27] criterion was taken into consideration, based on the number of inflammatory cells: if fewer than 100 (in 10 different fields magnified at 400 times), the inflammatory infiltration was considered to be *mild*; if there were between 100 and 500 inflamed cells, *moderate*; and if there were over 500 inflamed cells, this would be considered *severe* or *intense*. Also taken into consideration were the vascular neoformation and fibroblastic proliferation alongside the implant, known as a Spangberg irritation signal.[28]

RESULTS

1 Day

In Group I (saline), a thick band occupied by degraded cells, most notably polymorphonuclear neutrophils and macrophage, were seen in contact with the light of the tube (ie, the empty region inside the tube), and in deeper areas, neoformed capillaries were evidenced. In Group II (bupivacaine), an elevated number of polymorphonuclear neutrophils were observed in contact with the light of the tube, and they could also be observed in deeper areas (Figure 2). In this period, in Group III (articaine), a thin band of necrotic tissue followed by areas of inflammatory exudate was observed (Figure 3). In Group IV (lidocaine), an extensive area occupied by a moderate number of polymorphonuclear leucocytes, macrophages, and some lymphocytes could be observed. In Group V (mepivacaine), a thick band occupied by an elevated number of polymorphonuclear neutrophils and macrophages, as well as more distant neoformed capillaries, could be seen.

2 Days

After two days, in Group I (saline), an extensive band of neoformed connective tissue could be observed. During this period, in Group II (bupivacaine), degenerated tissue could be seen in contact with the light of the tube, followed by extensive areas with inflammatory exudate and rare fibroblasts. In Group III (articaine), a thin band occupied by a discrete number of neutrophils and macrophages could be observed in contact with the tube (Fig-

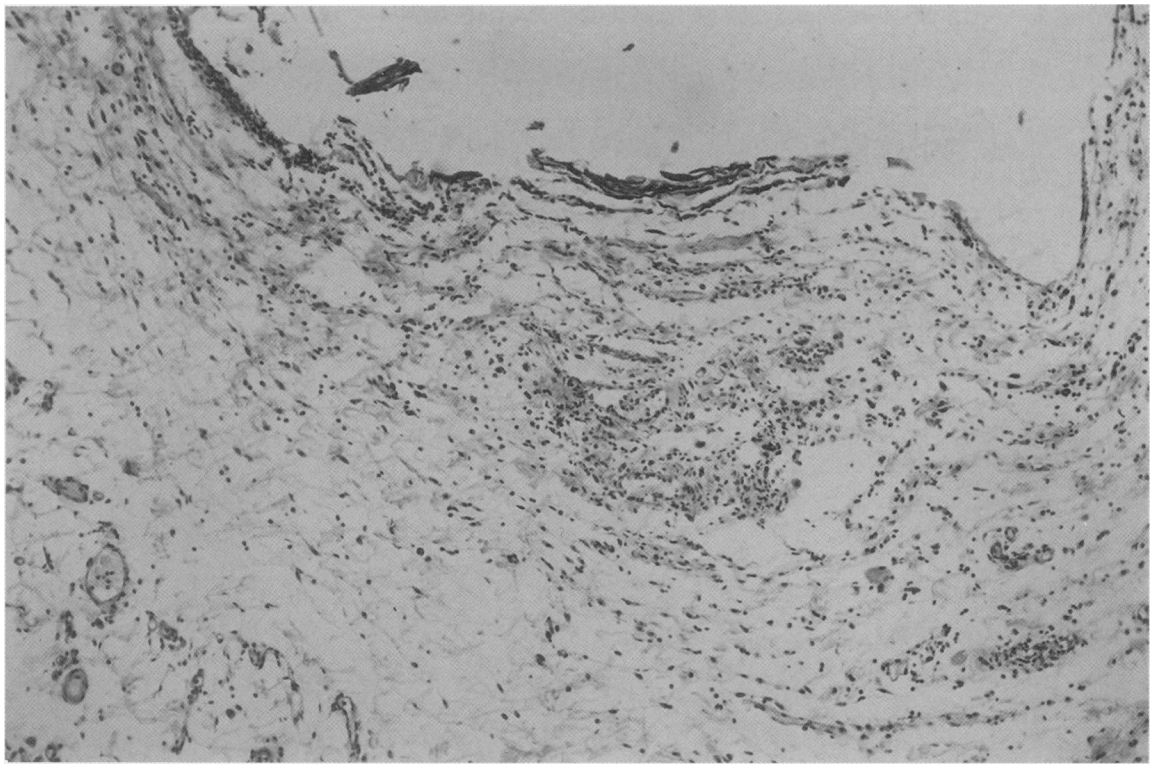

Figure 3. Group III (articaine). 1 day. A thin band of necrotic tissue in contact with the light of the tube was observed (HE; original magnification ×63).

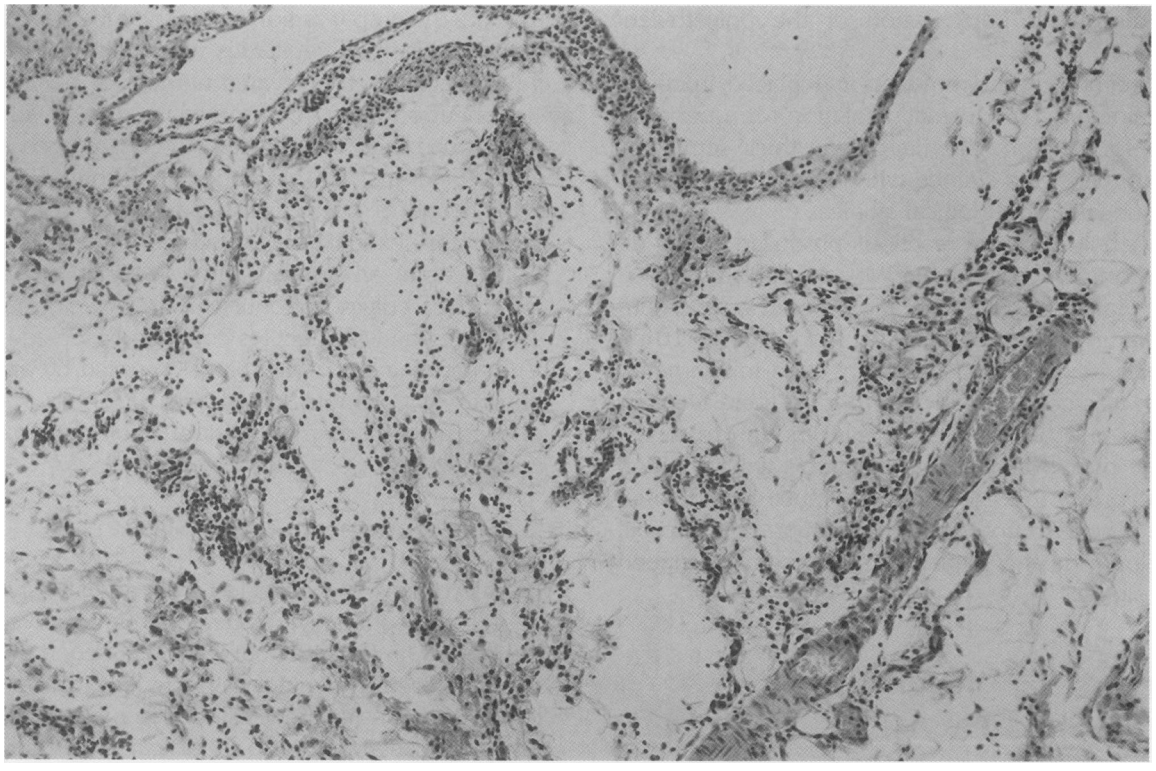

Figure 4. Group III (articaine). 2 days. A thin band occupied by a discrete number of neutrophils and macrophages was observed (HE; original magnification ×63).

ure 4). Rare fibroblasts were evidenced in the distant regions. In Group IV (lidocaine), a moderate number of neutrophils and macrophages could be evidenced. Below were some neoformed capillaries. In Group V (mepivacaine), in addition to the light of the tube, degenerated tissue could be observed. In deep regions, neoformed capillaries alongside some fibroblasts could be observed (Figure 5).

5 Days

Group I (saline) presented a discrete band of degenerated cells (Figure 6). Poorly organized, neoformed connective tissue could also be observed. In Group II (bupivacaine), alongside the light from the tube, a thick layer occupied by degenerated neutrophils and macrophages remained (Figure 7). Afterward, a moderate band of fibroblasts and neoformed capillaries could be observed. Then, in Group III (articaine), a thin band occupied by degenerated cells followed by a layer of connective tissue rich in fibroblasts and capillaries was evidenced. In very distant areas of Group IV (lidocaine), poorly organized connective tissue could be observed (Figure 8). In Group V (mepivacaine), near as well as deep into the tube, we found some macrophages and lymphocytes with a moderate number of fibroblasts and collagen fibers.

10 Days

In the last period, it was already possible in Group I (saline) to verify an extensive quantity of neoformed connective tissue rich in fibroblasts. In Group II (bupivacaine), along the tubular opening, the presence of neutrophils and macrophage could still be observed, and afterward a great amount of poorly organized neoformed connective tissue (Figure 9). In Group III (articaine), an extensive poorly organized neoformed connective tissue can be seen (Figure 10). Group IV (lidocaine), as well as Group III (articaine), presented a thin band occupied by degenerated cells. However, immediately below, neoformed connective tissue with some fibroblasts positioning parallel to the surface of the tubular opening could be observed. In Group V (mepivacaine), degenerated cells and a thick band of connective tissue with an elevated number of fibroblasts, some of which were laid out parallel to the light of the tube, was evidenced (Figure 11).

For interpretation and quantification of the results, and a comparison among the groups, the obtained tissue reactions are represented in 2 tables (Tables 1 and 2).

DISCUSSION

The present study evaluated tissue reactions to different anesthetic solutions implanted subcutaneously in rats. It is known that the tissue reactions in the subcutaneous region vary according to the quality and quantity of the material that is implanted.[28–30]

The factors that affect connective tissue reactions may be minimized if the recommendations presented in 1972 by the American Dental Association through the Council on Dental Materials and Devices are employed.[31] These recommendations were followed in this study. Like Carvalho et al[13] in 1976, we believe that the use of polyethylene tubes for reaction tests to solutions subcutaneously implanted in rats not only allows for a more prolonged contact of the anesthetic with the tissues but also favors the locating of the material and therefore the area that will be studied microscopically.

In 1981, Pullin[32] tested the use of polyethylene tubes filled only with absorbent paper cones. According to the author, the morphological aspect of the connective tissue in the light of the tube and its lateral walls was not the same. This result was mainly due to the greater smoothness of the material on the lateral surface. In 1966, Torneck[29] demonstrated that empty spaces in the polyethylene tubes do not cause irritation to the adjacent connective tissue, making these materials acceptable for this tissue. However, in 1977, Bernabé[33] admitted that the physical irritation at the extremities of the polyethylene tubes may be caused by the movement of the tube in the subcutaneous space.

In our study, during the first period of animal sacrifice it was possible to verify the presence of an intense acute inflammatory infiltrate in the region near the light of the tubes in all groups, with the exception of group III (articaine). In the area distant from the tube, acute infiltrate was considered to be mild in Groups I (saline), III (articaine), IV (lidocaine), and V (mepivacaine), and intense in Group II (bupivacaine). In our study, the tissue reaction by Group I (0.9% sodium chloride solution) proved to be mild to moderate near the light of the tube. Some authors report that already on the first postoperative day, the sodium chloride solution presents more favorable results than the other tested solutions.[13,34,35] We believe that this result can be attributed to a possible methodological difference. In contrast to these results, group I (saline) presented early reactions of vascular neoformation and fibroblastic proliferation of equal importance to the repair.

The intensity of the inflammatory infiltrate verified with Group IV (lidocaine) was similar to the results reported in the literature.[13,32] Because of these mild tissue reactions, the lidocaine solution is considered to be a standard comparison solution among the local anes-

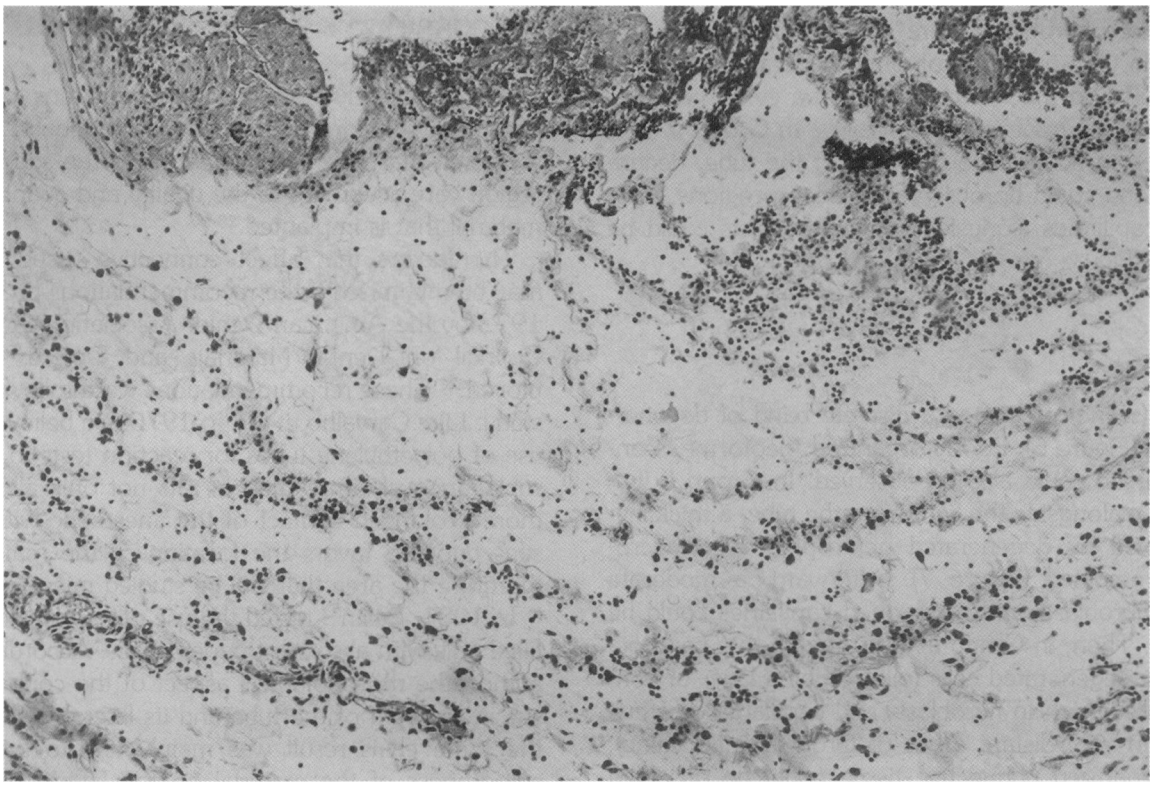

Figure 5. Group V (mepivacaine). 2 days. In addition to the light from the tube, degenerated tissue, including degenerated cells, was observed (HE; original magnification ×63).

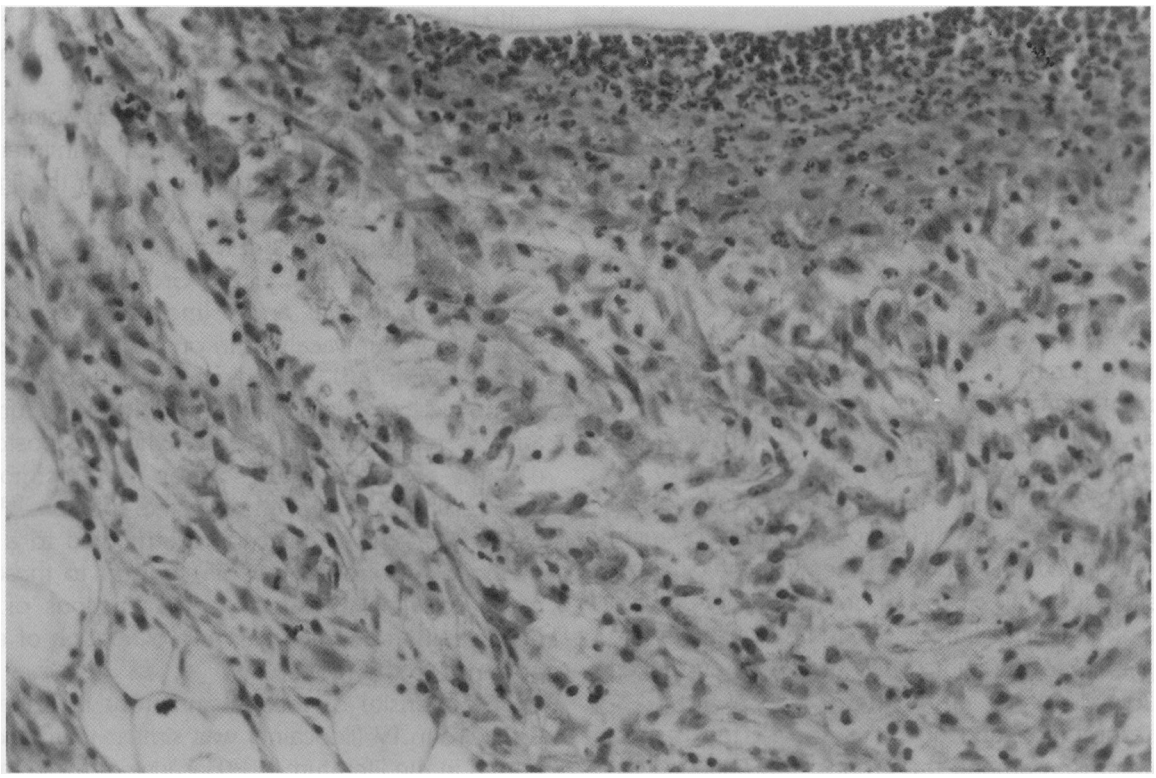

Figure 6. Group I (saline). 5 days. Immediately below the light of the tube, an elevated number of fibroblasts, some lymphocytes, and macrophages were observed (HE; original magnification ×160).

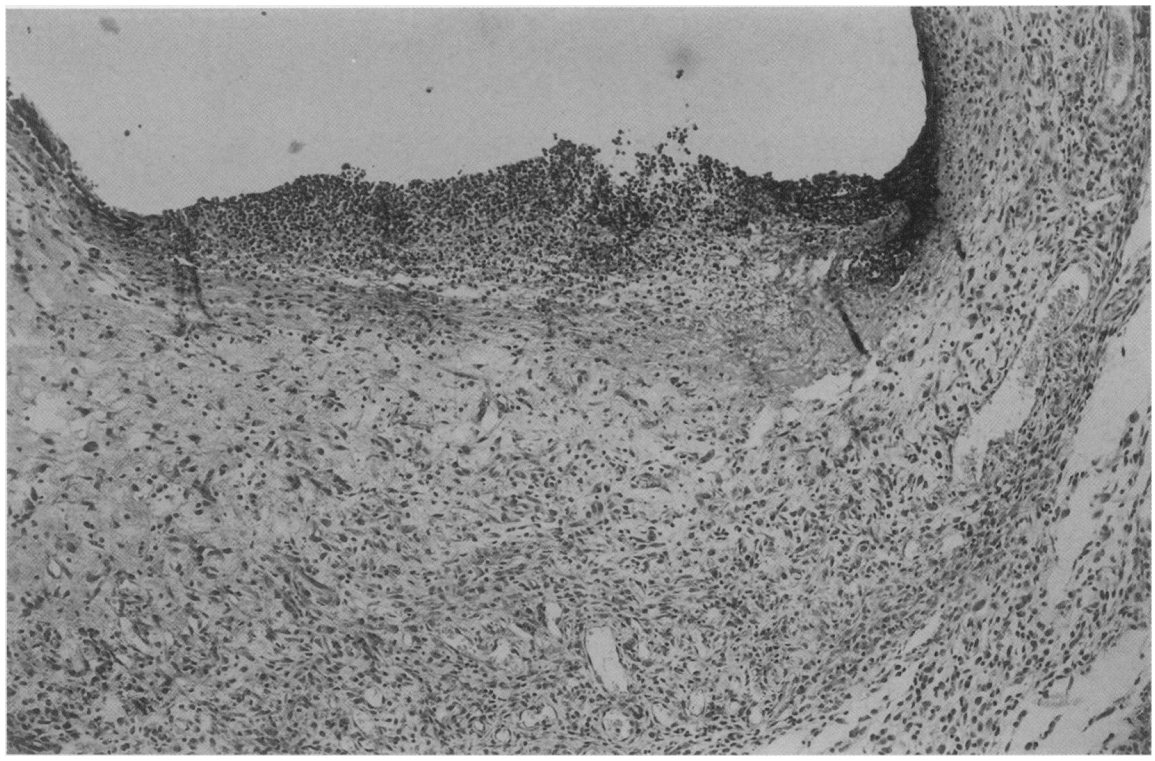

Figure 7. Group II (bupivacaine). 5 days. A thick layer occupied by cell remains, most notably polymorphonuclear neutrophils was observed (HE; original magnification ×63).

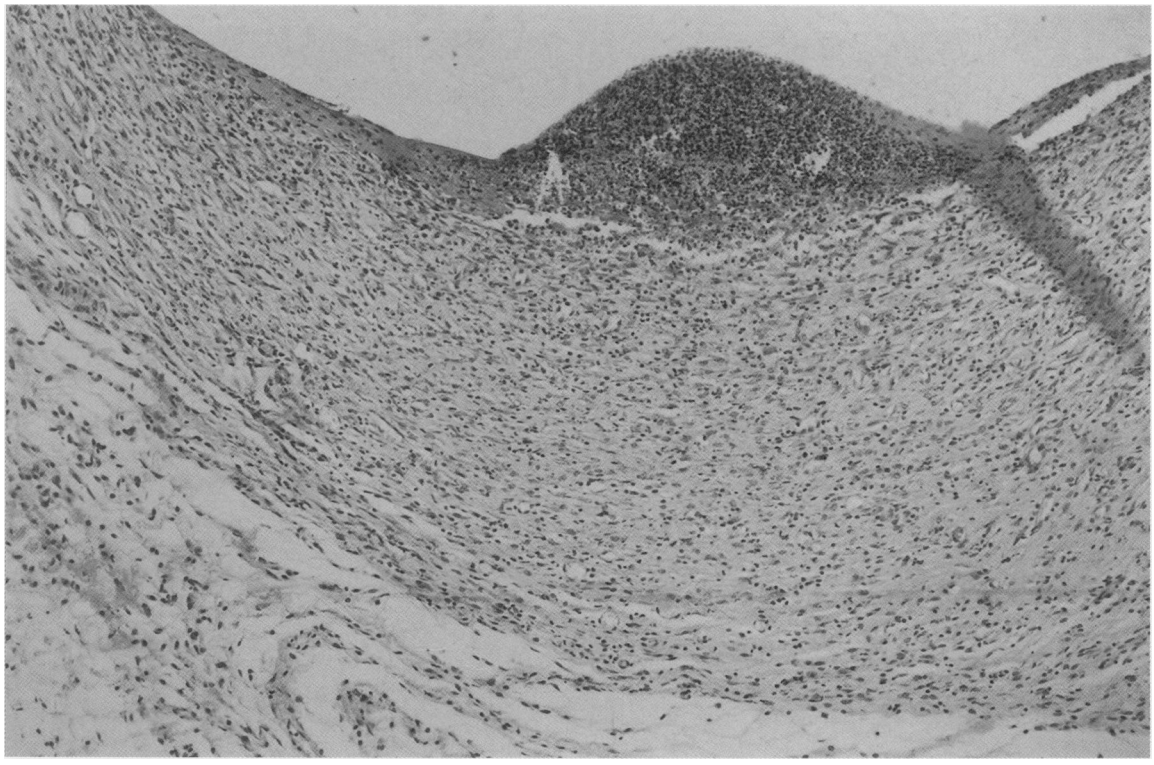

Figure 8. Group IV (lidocaine). 5 days. An extensive band of poorly organized connective tissue in deep areas was observed (HE; original magnification ×63).

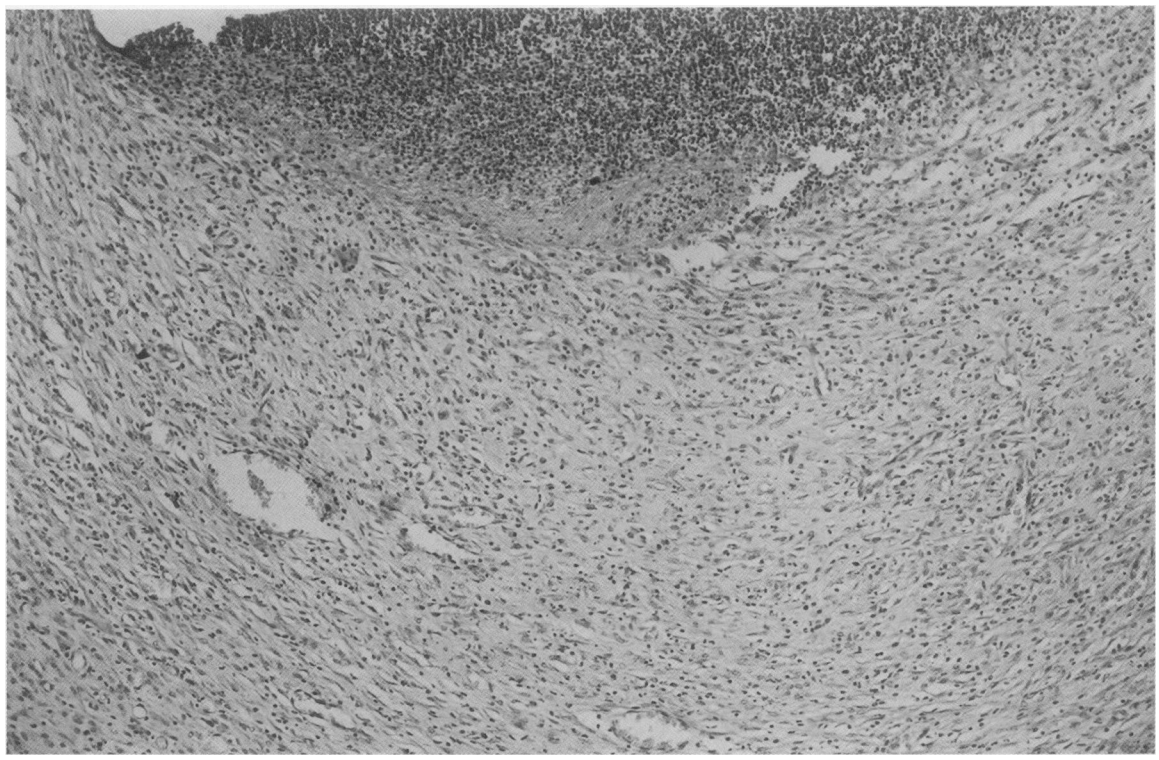

Figure 9. Group II (bupivacaine). 10 days. Poorly organized connective tissue infiltrated by neutrophils and macrophages can be noted along the tubular opening (HE; original magnification ×63).

Figure 10. Group III (articaine). 10 days. Below the tubular opening, an extensive layer of poorly organized neoformed connective tissue is observed (HE; original magnification ×63).

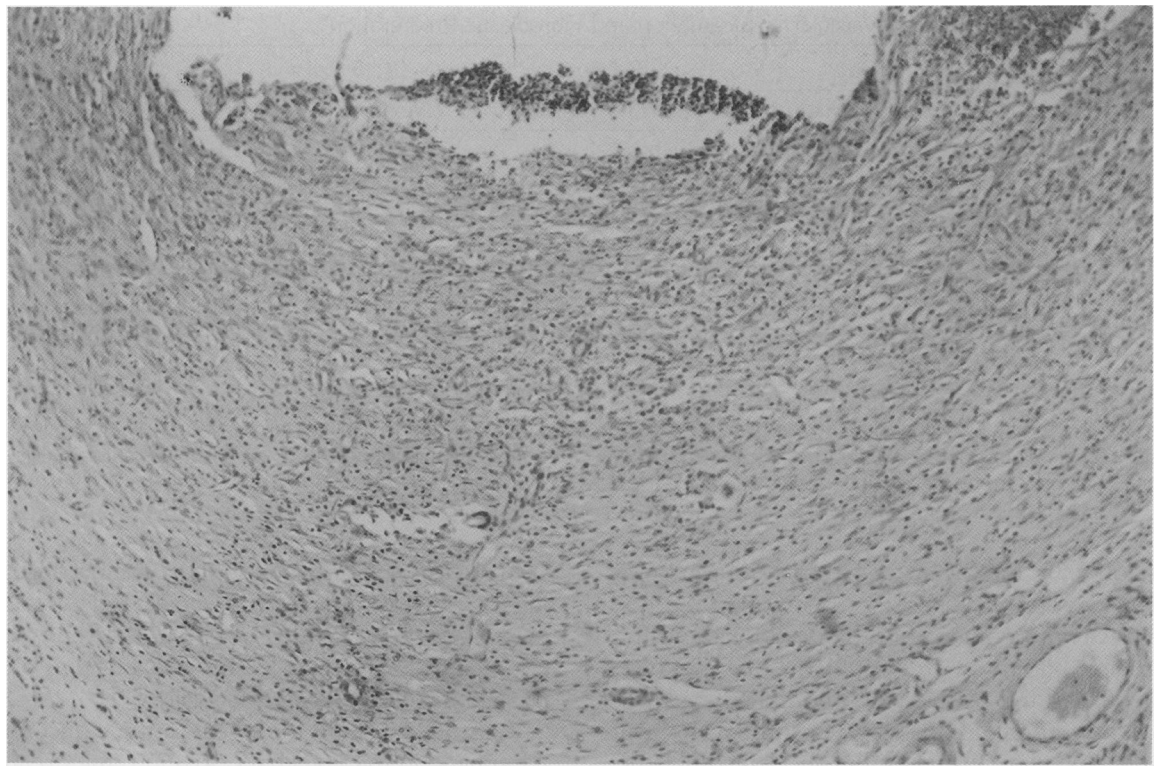

Figure 11. Group V (mepivacaine). 10 days. A thick band of connective tissue below the tubular opening, with an elevated number of fibroblasts, is observed (HE; original magnification ×63).

thetics. According to Saad Neto et al[36] in 1982, 2% lidocaine without a vasoconstrictor provides a better result because of its pharmacological characteristics. This presents a slight vasodilator effect, which induces a more rapid absorption and elimination of the anesthetic from the region. However, in 1976, Carvalho et al[13] considered the lidocaine to be the solution that caused the least tissue irritation, even with a vasoconstrictor. In 1972, Benoit and Belt[14] verified in rats that the lidocaine solution produced necrosis of the skeletal musculature; however, of the tested anesthetic solutions, it produced the most superficial necrosis, which allowed for earlier tissue repair.

Lidocaine has undergone several clinical studies. In 1999, Kramp et al[24] verified that 2% lidocaine with 1 : 100,000 epinephrine produced greater discomfort during and after the injection of the local anesthetic in humans when compared with 4% prilocaine without vasoconstrictor. In the same year, Jorkjend and Skoglund[26] compared 2% lidocaine without vasoconstrictor to the

Table 1. Location and Intensity of the Inflammatory Infiltrate*

		1 Day		*2 Days*		*5 Days*		*10 Days*	
Groups		*Near*	*Distant*	*Near*	*Distant*	*Near*	*Distant*	*Near*	*Distant*
I (SAL)	A	+++	++	+++	—	+	—	+	—
	C	++	+	—	+	—	+	—	+
II (BUP)	A	+++	+++	+++	—	+++	—	++	—
	C	+	+	—	++	++	+	—	++
III (ART)	A	++	+	+	—	++	—	+	—
	C	—	+	+	++	++	+	—	++
IV (LIDO)	A	++	—	++	—	+++	—	+	—
	C	+	+	+	++	—	++	—	+
V (MEP)	A	+++	—	+	++	—	—	+	—
	C	—	+	+	++	+	—	—	++

* For comparison, the histological data were considered to be mild (+), moderate (++) and intense (+++), as per respective magnitudes. The absence of these elements is noted by the sign (—). SAL indicates saline; BUP, bupivacaine; ART, articaine; LIDO, lidocaine; MEP, mepivacaine; A, acute; and C, chronic.

Table 2. Location and Intensity of Vascular Neoformation and Fibroblastic Proliferation*

		1 Day		*2 Days*		*5 Days*		*10 Days*	
Groups		*Near*	*Distant*	*Near*	*Distant*	*Near*	*Distant*	*Distant*	Near
I (SAC)	V	—	+	—	+	—	++	—	++
	F	—	—	—	+	—	+++	—	+++
II (BUP)	V	—	—	—	++	—	+++	—	++
	F	—	—	—	+	—	+++	—	+++
III (ART)	V	—	—	—	—	—	+++	—	++
	F	—	—	—	+	—	+++	—	++
IV (LIDO)	V	—	—	—	++	—	++	—	+++
	F	—	—	—	—	—	++	—	+++
V (MEP)	V	—	+	—	+	—	+	—	+++
	F	—	—	—	+	++	++	—	+++

* For comparison, the histological data were considered to be mild (+), moderate (++) and intense (+++), as per respective magnitudes. The absence of these elements is noted by the sign (—). SAL, indicates saline; BUP, bupivacaine; ART, articaine; LIDO, lidocaine; MEP, mepivacaine; V, vascular; and F, fibroplastic.

same solution with 1 : 80,000 epinephrine. They demonstrated, under statistical analysis, that during the first hours, pain was more intense in the group without the vasoconstrictor, but in the group in which the vasoconstrictor was added the pain was more intense up to 11 hours after the surgery. In this way, we agree with those experimental and clinical studies that show favorable results in the cases where no vasoconstrictor was used in combination with lidocaine. In the present study, this group was used as a pattern and as a comparison with other anesthetics, even knowing that the clinical duration of their effect is minimal.

The solutions presented by Groups II (bupivacaine), III (articaine), and V (mepivacaine) were chosen for evaluation because all the effects of these solutions on tissues are not known. Furthermore, they are anesthetic solutions that present different concentrations of anesthetic salts and vasoconstrictors.

In the initial periods of the solution, Group III (articaine) presented a more discrete inflammatory infiltrate when compared with the solutions from Groups II and V. These findings confirm the 1986 results of Bennett,[17] who reported that the anesthetic solution's degree of systemic toxicity does not always coincide with the local tissue irritability provided by this solution. The articaine solution (Group III) has a concentration of 40mg/mL, which is the highest of the tested anesthetics, and consequently is the most toxic.[8,11] In the final periods of the study, the intensity of the inflammatory infiltrate of Group III's articaine solution was similar to that of the other groups.

In the initial and final periods, Group II's bupivacaine solution demonstrated greater local irritability of the region when compared with the tested solutions. In this same group, vascular neoformation and fibroblastic proliferation proved to be practically the same as in the other solutions.

Studies report that the longest lasting anesthetic solutions, such as bupivacaine, cause skeletal musculature atrophy at the site of the injection, reaching 70% of the muscle volume in some cases. This atrophy was verified morphologically and histologically.[37–39]

It is known that the greater the concentration of adrenaline in the anesthetic solution, the greater the local irritability of the solution.[13,39] Bupivacaine solution tested in this study had the lowest concentration of adrenaline among the compared substances, so we believe this substance was not the one that induced the local irritation caused by this anesthetic solution. Furthermore, this solution had a low concentration of anesthetic salts.

Group V (mepivacaine) presented generally good tissue acceptability, similar to the solutions from Groups I (saline) and IV (lidocaine). In the 5- and 10-day periods, respectively, this solution had an inflammatory infiltrate intensity that was less than or equal to that of the other substances.

Mepivacaine solution was tested by Benoit and Belt[14] (1972), and they verified that this solution produced necrosis in the skeletal musculature at the point of injection, with this effect being followed by complete regeneration, although in periods that took longer than those involving lidocaine. Delay in the alveolar repair process chronology in rats was recently attributed to the use of 2% mepivacaine with 1 : 100,000 adrenaline.[41]

The presence of a greater concentration of vasoconstrictors in Groups III (articaine) and V (mepivacaine) did not cause greater tissue irritation with these solutions. However, in 1982, Saad Neto et al[33] reported that the greater the concentration of vasoconstrictors of the catecholamine type, such as adrenaline in the anesthetic solutions, the greater the predisposition for accentuated tissue irritability. This irritability may also be seen when greater concentrations of adrenaline

were used in association with the lidocaine and prilocaine solutions.[23–26,40,42]

The occurrence of pain after the analgesic effect of the anesthetic has passed may be associated with the presence of vasoconstrictors. Kramp et al[24] and Jorkjend and Skoglund[26] have demonstrated that pain intensity was greatly accentuated in groups in which solutions containing vasoconstrictors were used.

These experimental and clinical results lead us to believe that the anesthetic solutions that contain vasoconstrictors in their composition are more irritating to the tissue, and this tissue irritability may be the postanesthetic discomfort factor. Consequently, the use of anesthetic solutions that contain a high concentration of vasoconstrictors will invariably make this solution more irritant locally, and many times systemically. However, as can be verified in this study, these substances are not specifically the only ones that have the tissue irritability effect.

We agree with Carvalho and Okamoto[43] that the tissue irritation alone provoked by anesthetics and detected at a histological level would already be sufficient to cause discrete postanesthetic pain. Furthermore, other local complications associated with the anesthetic solutions may have their origin in tissue irritability caused by these substances in the tissues, such as trismus, tissue necrosis of the mucosa, and neurological disorders.[1,43,44]

Because of the present results, we agree with other authors that the greater or lesser incidence of tissue irritability is caused by the sum of the components that make up the anesthetic solution rather than a specific component of the drug.[13,36,43]

CONCLUSION

On the basis of the analysis of the histological results obtained from this study, we can conclude that (a) the tested anesthetic solutions presented different tissue reactions; (b) the bupivacaine group presented the most intense inflammatory reaction; (c) the articaine and mepivacaine groups generated similar inflammatory reactions; and (d) the lidocaine group presented the least intense inflammatory reaction.

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